

REQUEST TO REOPEN PROSECUTION UNDER 37 C.F.R. § 41.50(a) AND AMENDMENT UNDER 37 C.F.R. § 1.111
Serial Number: 09/458,862
Filing Date: December 10, 1999
Title: COMPOSITIONS AND METHODS FOR CRYOPRESERVATION OF PERIPHERAL BLOOD LYMPHOCYTES

Page 2
Dkt: 600451US1

In the Claims

Please amend the claims as follows:

1. (Currently Amended) A cryopreservation medium comprising a balanced electrolyte solution, incorporating at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v, a cryoprotective agent that penetrates the cell membrane, which agent is present in an amount of 1% w/v to 40% w/v, and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan, a biological or functional equivalent thereof, in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified *ex vivo*.
2. (Original) The cryopreservation medium of claim 1 wherein the cells are peripheral blood lymphocytes.
3. (Original) The cryopreservation medium of claim 1 that comprises arabinogalactan.
4. (Canceled)
5. (Currently Amended) The cryopreservation medium of claim 4 1 wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycol.
6. (Original) The cryopreservation medium of claim 1 further comprising a cryoprotective agent other than arabinogalactan or a biological or functional equivalent thereof which does not penetrate the cell membrane.
7. (Original) The cryopreservation medium of claim 1 which does not comprise protein.
8. (Original) The cryopreservation medium of claim 1 which is infusible.

REQUEST TO REOPEN PROSECUTION UNDER 37 C.F.R. § 41.50(a) AND AMENDMENT UNDER 37 C.F.R. § 1.111
Serial Number: 09/458,862
Filing Date: December 10, 1999
Title: COMPOSITIONS AND METHODS FOR CRYOPRESERVATION OF PERIPHERAL BLOOD LYMPHOCYTES

Page 3
Dkt: 600.451US1

9-10. (Canceled)

11. (Original) The cryopreservation medium of claim 1 wherein the cells are human cells.

12. (Original) The cryopreservation medium of claim 1 wherein the cells are non-human vertebrate cells.

13. (Canceled)

14. (Currently Amended) A composition suitable for administration to a human, comprising a suspension of cells in a cryopreservation medium comprising a balanced electrolyte solution, ~~incorporating at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v, and a cryoprotective agent that penetrates the cell membrane, wherein arabinogalactan, or a biological or functional equivalent thereof, is present in an amount of 1% w/v to 40% w/v, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified ex vivo, or a combination thereof,~~ and wherein the medium does not comprise dimethylsulfoxide or serum.

15. (Canceled)

16. (Previously Presented) The composition of claim 14 wherein the cells are peripheral blood lymphocytes.

17. (Currently Amended) The composition of claim 14 wherein ~~at least one of the cryoprotective agents is the medium comprises~~ arabinogalactan.

18. (Canceled)

REQUEST TO REOPEN PROSECUTION UNDER 37 C.F.R. § 41.50(e) AND AMENDMENT UNDER 37 C.F.R. § 1.111
Serial Number: 09/458,862
Filing Date: December 10, 1999
Title: COMPOSITIONS AND METHODS FOR CRYOPRESERVATION OF PERIPHERAL BLOOD LYMPHOCYTES

Page 4
Dkt: 600.451US1

19. (Previously Presented) The composition of claim 14 wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycol.
20. (Previously Presented) The composition of claim 14 further comprising a cryoprotective agent other than arabinogalactan or a biological or functional equivalent thereof which does not penetrate the cell membrane.
21. (Previously Presented) The composition of claim 14 which does not comprise protein.
22. (Previously Presented) The composition of claim 14 which is infusible.
23. (Canceled)
24. (Previously Presented) The composition of claim 14 wherein the cells are human cells.
25. (Canceled)
26. (Currently Amended) A method for preserving cells comprising:
 - (a) contacting cells with a cryopreservation medium comprising a balanced electrolyte solution, and at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v, and a cryoprotective agent that penetrates the cell membrane, to yield a cell suspension, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan, a biological or functional equivalent thereof, in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified *ex vivo*; and
 - (b) freezing the cell suspension to yield a frozen cell suspension.

REQUEST TO REOPEN PROSECUTION UNDER 37 C.F.R. § 41.50(a) AND AMENDMENT UNDER 37 C.F.R. § 1.111
Serial Number: 09/458,862
Filing Date: December 10, 1999
Title: COMPOSITIONS AND METHODS FOR CRYOPRESERVATION OF PERIPHERAL BLOOD LYMPHOCYTES

Page 5
Dkt: 600.451US1

27. (Original) The method of claim 26 further comprising thawing the frozen cell suspension under conditions that maintain cell viability.
28. (Original) The method of claim 26 wherein the cells are human cells.
29. (Canceled)
30. (Original) The method of claim 26 wherein the cells are peripheral blood lymphocytes.
31. (Currently Amended) A frozen composition comprising i) inorganic salts capable of maintaining physiological pH when in solution a balanced electrolyte solution, ii) at least one eryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v, iii) a cryoprotective agent that penetrates the cell membrane, and iv) freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the composition does not comprise dimethylsulfoxide or serum.
32. (Original) A frozen hematopoietic cell-containing composition made according to the method of claim 26.
33. (Original) The cryopreservation medium of claim 5 wherein the cryoprotective agent that penetrates the cell membrane is glycerol.
34. (Original) The cryopreservation medium of claim 33 wherein the concentration of glycerol is about 1% to about 3%.
35. (Previously Presented) The cryopreservation medium of claim 1 wherein the lymphocytes which are modified *ex vivo* are activated lymphocytes or genetically modified lymphocytes.

REQUEST TO REOPEN PROSECUTION UNDER 37 C.F.R. § 41.50(a) AND AMENDMENT UNDER 37 C.F.R. § 1.111
Serial Number: 09/458,862
Filing Date: December 10, 1999
Title: COMPOSITIONS AND METHODS FOR CRYOPRESERVATION OF PERIPHERAL BLOOD LYMPHOCYTES

Page 6
Dkt: 600451US1

36. (Previously Presented) The composition of claim 14 or 31 wherein the lymphocytes which are modified *ex vivo* are activated lymphocytes or genetically modified lymphocytes.

37. (Previously Presented) A cryopreservation medium comprising a balanced electrolyte solution, at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the balanced electrolyte solution is selected from the group consisting of lactated Ringer's solution, PlasmaLyte-A™, Normosol-R™, Veen-D™, Polysal®, and Hank's balanced salt solution.

38. (Previously Presented) The cryopreservation medium of claim 37 wherein the lymphocytes are peripheral blood lymphocytes.

39. (Previously Presented) The cryopreservation medium of claim 37 wherein the agent is arabinogalactan.

40. (Previously Presented) The cryopreservation medium of claim 37 further comprising a cryoprotective agent that penetrates the cell membrane.

41. (Previously Presented) The cryopreservation medium of claim 40 wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycol.

42. (Previously Presented) The cryopreservation medium of claim 37 further comprising a cryoprotective agent other than arabinogalactan or a biological or functional equivalent thereof which does not penetrate the cell membrane.

43. (Previously Presented) The cryopreservation medium of claim 37 which does not comprise protein.

REQUEST TO REOPEN PROSECUTION UNDER 37 C.F.R. § 41.50(a) AND AMENDMENT UNDER 37 C.F.R. § 1.111
Serial Number: 09/458,862
Filing Date: December 10, 1999
Title: COMPOSITIONS AND METHODS FOR CRYOPRESERVATION OF PERIPHERAL BLOOD LYMPHOCYTES

Page 7
Dkt: 600.451 US1

44. (Previously Presented) The cryopreservation medium of claim 37 which is infusible.

45-46. (Canceled)

47. (Previously Presented) The cryopreservation medium of claim 37 wherein the cells are human cells.

48. (Previously Presented) The cryopreservation medium of claim 37 wherein the cells are non-human vertebrate cells.

49. (Previously Presented) The method of claim 26 wherein the medium comprises arabinogalactan.

50. (Canceled)

51. (Currently Amended) The method of claim 50 26, 57 or 58 wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycol.

52. (Currently Amended) The method of claim 26, 57 or 58 wherein the lymphocytes which are modified *ex vivo* are activated lymphocytes or genetically modified lymphocytes.

53. (Currently Amended) A cryopreservation medium comprising a balanced electrolyte solution, incorporating at least one cryoprotective agent that is arabinogalactan, which is present in an amount of 1% w/v to 40% w/v, a cryoprotective agent that penetrates the cell membrane, and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified *ex vivo*.

REQUEST TO REOPEN PROSECUTION UNDER 37 C.F.R. § 41.50(a) AND AMENDMENT UNDER 37 C.F.R. § 1.111
Serial Number: 09/458,862
Filing Date: December 10, 1999
Title: COMPOSITIONS AND METHODS FOR CRYOPRESERVATION OF PERIPHERAL BLOOD LYMPHOCYTES

Page 8
Dkt: 600.451US1

54. (Currently Amended) A cryopreservation medium comprising a balanced electrolyte solution, incorporating at least one cryoprotective agent that is arabinogalactan, which is present in an amount of 1% w/v to 40% w/v, glycerol in amount of 0.5% to about 20%, and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified *ex vivo*.

55. (Currently Amended) A frozen composition comprising i) inorganic salts capable of maintaining physiological pH when in solution a balanced electrolyte solution, ii) at least one cryoprotective agent that is arabinogalactan in an amount of 1% w/v to 40% w/v, iii) a cryoprotective agent that penetrates the cell membrane, and iv) freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the composition does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the composition results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified *ex vivo*.

56. (Currently Amended) A frozen composition comprising i) inorganic salts capable of maintaining physiological pH when in solution a balanced electrolyte solution, ii) at least one cryoprotective agent that is arabinogalactan in an amount of 1% w/v to 40% w/v, iii) glycerol in amount of 0.5% to about 20%, and iv) freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the composition does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the composition results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified *ex vivo*.

57. (Currently Amended) A method for preserving cells comprising: freezing a cell suspension comprising cells and a cryopreservation medium comprising a balanced electrolyte solution, arabinogalactan in an amount of 1% w/v to 40% w/v, and glycerol in amount of 0.5% to

REQUEST TO REOPEN PROSECUTION UNDER 37 C.F.R. § 41.50(a) AND AMENDMENT UNDER 37 C.F.R. § 1.111
Serial Number: 09/458,862
Filing Date: December 10, 1999
Title: COMPOSITIONS AND METHODS FOR CRYOPRESERVATION OF PERIPHERAL BLOOD LYMPHOCYTES

Page 9
Dkt: 600451US1

about 20%, to yield a cell suspension, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified *ex vivo*.

58. (Currently Amended) A method for preserving cells comprising:

(a) contacting cells with a cryopreservation medium comprising a balanced electrolyte solution, and at least one cryoprotective agent that is arabinogalactan, in an amount of 1% w/v to 40% w/v, and a cryoprotective agent that penetrates the cell membrane, to yield a cell suspension, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, and wherein the medium does not comprise dimethylsulfoxide or serum; and

(b) freezing the cell suspension at a cooling rate of about 1° to about 10° C/minute to yield a frozen cell suspension.

59. (New) The medium of claim 1, 37, 53 or 54 wherein the post-thaw survival rate is at least about 40%.

60. (New) The method of claim 26, 57 or 58 wherein the post-thaw survival rate is at least about 40%.

61. (New) A cryopreservation medium comprising a balanced electrolyte solution, arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v, and a cryoprotective agent that penetrates the cell membrane.

62. (New) The medium of claim 61 wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycerol.

63. (New) The medium of claim 62 wherein glycerol is about 1% to about 5%.

BEST AVAILABLE COPY

REQUEST TO REOPEN PROSECUTION UNDER 37 C.F.R. § 41.50(a) AND AMENDMENT UNDER 37 C.F.R. § 1.111
Serial Number: 09/458,862
Filing Date: December 10, 1999
Title: COMPOSITIONS AND METHODS FOR CRYOPRESERVATION OF PERIPHERAL BLOOD LYMPHOCYTES

Page 10
Dkt: 600.451US1

64. (New) The cryopreservation medium of claim 61 wherein the arabinogalactan is about 10% w/v to about 30% w/v.